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Intestinal Absorption and Esterification of ^{14}C-labeled Fatty Acids in Man *

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Investigations in which the lymph from the intestine has been collected have provided fundamental information on the subject of fat absorption (1, 2). In their classical studies on a patient with a lymph fistula, Munk and Rosenstein obtained good experimental evidence that fed triglycerides as well as free fatty acids are transported via the lymphatic pathway mainly as triglycerides (1). Since the introduction of the tracer technique, this transport route for saturated and unsaturated long chain fatty acids has been well established in both animal experiments (3, 4) and human investigations (5–7). The major part of the absorbed fatty acids is transported via the thoracic duct, in the chylomicron fraction of the lymph, mainly as triglycerides but to a small extent also as cholesterol esters and phospholipids (5, 6, 8). In these studies a single labeled fatty acid has been fed and its distribution measured among the lipid classes of the thoracic duct lymph.

Studies on the specificity of fatty acid esterification during fat absorption have been reported by Karmen, Whyte, and Goodman (9, 10). In these studies, mixtures of several labeled fatty acids were fed to rats with thoracic duct fistulas, and the distribution of labeled and unlabeled fatty acids was measured for several different lymph lipids.

Chylomicron cholesterol ester formation showed a specificity for oleic acid relative to palmitic, stearic, and linoleic acids. Chylomicron triglyceride formation showed no specificity for one fatty acid relative to another with the exception of slight discrimination against stearic acid. The formation of chylomicron lecithin showed a marked relative specificity for stearic acid and a lesser specificity for linoleic acid. In a similar investigation (11) conducted in man we reported preliminary results that there is also a preferential incorporation of oleic acid into the cholesterol esters, relative to other fatty acids tested.

In a continuation of earlier investigations on the transport of lipids via the thoracic duct in man, we have studied the distribution of mass and radioactivity in certain major lipid fractions of the chyle after feeding mixtures of labeled fatty acids. The results of these studies form the subject of this report.

Methods

Labeled materials. Palmitic acid-1-^{14}C, stearic acid-1-^{14}C, oleic acid-1-^{14}C, linoleic acid-1-^{14}C, and tripalmitin-1-^{14}C were obtained commercially.[1] Analysis for radioactive impurities by gas-liquid radiochromatography as described below indicated each fatty acid to be more than 99% pure.

Metabolic design. The studies to be described were performed on patients with diagnosed cancer of different types (see the case reports in the Appendix). The thoracic duct was cannulated by a method similar to that described by Shafiroff and Kau (12). The patients were fasted overnight before surgery. After the cannulation of the thoracic duct the lymph was collected for several hours while the patients were fasting. In Patients 1, 2, and 4 the liquid formula meal was administered 7, 5, and 5 hours, respectively, after the operation. Patient 3 was fed a general mixed diet after the operation. She was not allowed to eat anything after 9 p.m. At 11 a.m. the following day, when the patient had been fasting 14 hours, she was fed the formula meal. Drinking the formula took the patients only a few minutes. The liquid formula meals (Tables I and II) contained labeled fatty acids as free fatty acids (Patients 1, 3, and 4) or as triglyceride (Patient 2). The patients were treated throughout the

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[1] Radiochemical Centre, Amersham, England.

TABLE I

Composition of the liquid formula given to patients

Patient	Type of fat	Glucose		Protein egg white	Water to make	Isotope fed	Amount
		g	g	g	g		μc
1	Olive oil	22	41.5	37.5	251.0	Oleic acid-1-^{14}C	15
2	Rape seed oil	22	41.5	37.5	251.0	Tripalmitin-1-^{14}C	15
3	Palmitic acid	2				Palmitic acid-1-^{14}C	15
	Oleic acid	2	20.75	18.75	120.5	Oleic acid-1-^{14}C	15
	Linoleic acid	2				Linoleic acid-1-^{14}C	15
4	Palmitic acid	1.5				Palmitic acid-1-^{14}C	11.25
	Stearic acid	1.5	11.3	10.2	98.5	Stearic acid-1-^{14}C	11.25
	Oleic acid	1.5				Oleic acid-1-^{14}C	11.25
	Linoleic acid	1.5				Linoleic acid-1-^{14}C	11.25

investigation with intravenous infusions of glucose, saline, and albumin and were allowed to drink water, tea, and coffee. Electrolyte and water balance was controlled by analysis of the blood, lymph, and urine. The lymph was collected continuously (in serial 1-hour fractions) directly into heparinized containers kept in an icebox at + 4° C.

Lipid extraction and analysis. Extraction of lymph was carried out mainly by the method of Folch, Lees, and Sloane Stanley (13). Samples of the different lymph samples were extracted with 20 vol of chloroform-methanol 2:1 (vol/vol). After splitting the mixture into two phases, the chloroform phase was collected and evaporated to dryness *in vacuo* under a stream of nitrogen. The total lipid extract was dissolved in benzene and stored under nitrogen at − 15° C.

Samples of the benzene solutions were taken for determination of lipid weight with a micro-Cahn balance and for total cholesterol (14), lipid phosphorus (15), and triglyceride glycerol (16).

Silicic acid chromatography was employed to separate the lipid samples into cholesterol esters, glycerides, and phospholipid fractions (17, 18). Columns of 10 mm internal diameter were loaded with 2.5 g of silicic acid. The chromatographic load did not exceed 30 mg per g silicic acid. The sterol ester fraction was eluted with 250 ml benzene in hexane 15:85 (vol/vol). The purity of the cholesterol ester fraction was tested with thin-layer chromatography. In model experiments, cholesteryl-oleate-1-^{14}C was also used to check the efficiency of the separation of cholesterol esters from the triglycerides. The triglyceride fraction was then eluted with 250 ml benzene.

The column was next eluted with 250 ml chloroform, followed by elution of the total phospholipids with 100 ml methanol. The chloroform fraction contained free cholesterol and free fatty acids. The free fatty acids were not further analyzed.

After chromatography portions of the various fractions were taken for determination of weight on a micro-Cahn balance. The total recovery of radioactivity after column chromatography was in the range of 95 to 98%.

Thin-layer chromatography was used to check the composition of the effluent from the silicic acid column and in some cases for preparation of sufficient material for gas-liquid chromatography. Thin-layer plates of silica gel were developed by ascending solvent in tanks lined by absorbent paper. For optimal separation of cholesterol esters from triglycerides, light petroleum (bp 40 to 60° C)-diethyl ether-acetic acid 90:10:1 (vol/vol) was used, whereas for optimal separation of triglycerides, diglycerides, and monoglycerides, light petroleum-diethyl ether 70:30 (vol/vol) was used.

For phospholipid separation the chromatogram was developed in chloroform-methanol-water 65:30:5 (vol/vol).

After spraying one segment of the plates with rhodamine 6 G, the position of the lipids was located under ultraviolet light. The areas of silica gel containing the lipids were scraped off with a spatula, and the gel was extracted with chloroform, or in the case of phospholipids with methanol. This material was used for preparation of the methyl esters of the fatty acids and for determination of the radioactivity in the different lipid classes.

Gas-liquid chromatography. Portions of the cholesterol ester, triglyceride, and phospholipid fractions were

TABLE II

*Distribution of mass and radioactivity among the fatty acids of the test meals**

Patient	Mass distribution					Radioactivity distribution				Specific activities			
	16:0	18:0	18:1	18:2	22:1	16:0	18:0	18:1	18:2	16:0	18:0	18:1	18:2
			%				%			counts/sec/mg			
1	15.6	2.3	66.9	13.5				100				77.2	
2	1.5	0.5	7.1	10.4	67.6	100				1,234.0			
3	34.6		30.1	35.3		34.6		31.5	33.9	183.9		183.6	183.7
4	26.4	24.4	24.6	24.6		25.5	24.4	25.2	24.9	168.5	177.0	176.8	175.0

* Values are expressed as percentage of total fatty acid methyl esters and percentage of total radioactivity.

TABLE III

*Composition and distribution of radioactivity of human thoracic duct lymph lipids after oral administration of the test meals described in Table I**

Patient	Time after test meal of sample analysis	Per cent of lymph lipids found as			Per cent of lymph fat radioactivity found in		
		Cholesterol esters	Triglycerides	Phospholipids	Cholesterol ester fatty acids	Triglyceride fatty acids	Phospholipid fatty acids
	hours	%	%	%	%	%	%
1	7	1.5	89.7	8.8	0.4	98.1	1.5
2	6	2.5	86.3	11.2	0.5	97.1	2.4
3	8	7.7	80.3	12.0	1.5	96.6	1.9
4	7	6.5	80.3	13.2	1.7	96.2	2.1

* The lymph samples analyzed were collected at the peak of fat absorption.

saponified and the fatty acids extracted and esterified with diazomethane. Methyl esters were formed by transesterification with 1% sulfuric acid in methanol-benzene 2:1 (vol/vol). The distribution of fatty acid mass and radioactivity was simultaneously assayed as described below. Separate mass analyses were also performed with an analytical Pye argon gas chromatograph (strontium detector) to check the analyses of the fatty acid methyl esters. The sample was introduced into the column with the aid of a platina spiral as described earlier (19).

The column used, 1,200 mm long and 4 mm in internal diameter, contained 15% Egss-X, an organosilicon polyester packing,[2] on 100- to 120-mesh acid-washed and silane-treated Celite at a temperature of 195° C.

For simultaneous determination of mass and radioactivity a preparative Pye argon chromatograph equipped with a strontium detector and a pyrex glass effluent stream splitter was used (11, 20). The glass splitter was connected by silicon rubber fittings to a stainless steel tube filled in the first section with copper oxide and in the second section with reduced iron.

The stainless steel tube was heated to 800° C by means of an electronically controlled furnace, resulting in the complete combustion of fatty acid methyl esters during passage through the tube. The radioactivity of the $^{14}CO_2$ peak was assayed by a 10-ml internal flow proportional counter at room temperature with argon as the carrier gas and CO_2 added externally to give a final concentration of 4%. The details of calibration of the apparatus have been given previously (21). The column used was 1,200 mm in length and 10 mm in internal diameter and contained 14% polyethylene glycol succinate supported on Celite (100- to 120-mesh) treated as described above. The temperature of the column was 160° C. The output of both the strontium detector and the proportional counter was fed into a two-channel potentiometric recorder.[3] The areas were measured by cutting out peaks from the recorder chart and weighing the paper.

The radioassay of lipid fractions other than the gas-liquid chromatography effluents was performed by liquid scintillation counting with a Packard scintillation spectrometer. Samples were counted in 10 ml of a solution of 0.6% 2,5-diphenyloxazole and 0.03% 1,4-bis-2-(5-phenyloxazolyl) benzene in toluene.

[2] Applied Science Laboratories, State College, Pa.

[3] Model PWS, Texas Instruments, Houston, Texas.

Results

The composition of the dietary fatty acids and triglycerides and the distribution of the radioactivity among the fatty acids of the test meal in the different investigations are listed in Tables I and II. Table III presents the composition of the lymph lipids collected during the peak of fat absorption in each of the four patients.

The recovery of radioactivity in the lymph lipids of the various experiments after feeding the test meals was in the range of 40 to 59% of the administered radioactivity (Table IV).

There might be several causes for the variation among patients and the rather low recovery. There are considerable anatomical differences among the patients in the arrangement of lymph vessels (22), some of which might bypass the fistula. Anastomoses that make possible a bypassing of the thoracic duct might exist between lymph and blood vessels (23).

TABLE IV

Recovery of radioactivity in human thoracic duct lymph lipids after oral administration of the test meals described in Table I

Patient	Collection time of lymph after test meals	Per cent of administered radioactivity recovered in lymph fatty acids
	hours	%
1	20	40.7
2	22	59.7
3	13	49.5
4	16	46.4

TABLE V

Distribution of mass and radioactivity of fatty acids in cholesterol esters, triglycerides, and phospholipids of human thoracic duct lymph

Time after test meal	Fatty acids as per cent of total fatty acids			Per cent distribution of radioactivity among the fatty acids		
	Cholesterol ester fatty acids	Triglyceride fatty acids	Phospholipid fatty acids	Cholesterol ester fatty acids	Triglyceride fatty acids	Phospholipid fatty acids
hours	%	%	%	%	%	%
			Patient 3			
2	10.9	76.3	12.8	1.7	97.5	0.8
5	8.3	82.8	8.9	1.6	97.5	0.9
8	3.7	87.4	8.9	1.5	96.6	1.9
9	0.5	98.6	0.9	1.7	96.0	2.3
11	7.1	82.0	10.4	2.4	94.4	3.1
13	7.7	81.3	11.0	2.6	94.0	3.4
			Patient 4			
1	11.3	73.0	15.7	8.8	90.2	1.0
4	6.1	78.2	15.7	3.1	95.7	1.2
7	3.1	87.2	9.8	1.7	96.2	2.1
10	4.5	84.8	10.7	2.4	94.7	2.9
13	10.4	74.7	14.9	3.4	92.6	4.0
16	12.9	71.0	16.1	5.5	88.4	6.1

In Patients 1 and 2 labeled oleic acid and palmitic acid were fed as single labeled fatty acids, as free fatty acid, and as triglyceride, respectively (Tables I and II). Lymph samples analyzed at various time intervals with the aid of gas radiochromatography showed that all the radioactivity was associated with oleic acid or palmitic acid. This verified that there was no interconversion of fatty acids during the intestinal absorption process, thus providing a check on the validity of the technique used in Patients 3 and 4, where several fatty acids were fed simultaneously. The complete analyses of triglyceride, cholesterol ester, and phospholipid fractions of whole lymph from Patients 3 and 4 are presented in Tables V and VI, respectively. These Tables include the distribution of the total fatty acid mass and radioactivity in several lymph samples from each patient.

To investigate whether there was any variability in the absorption of palmitic, oleic, and linoleic acids, several lymph triglyceride samples at different time intervals were analyzed in Patient 3 (Table VI). No significant difference in the absorption of these fatty acids was observed during the time intervals studied (6 to 10 hours).

The concentration of triglycerides in the various lymph fractions together with the lymph volumes are given in Figure 1. The incorporation of the labeled fatty acids into the lymph triglycerides is also given. In this Figure the relative specific activity is expressed as the specific activity found in per cent of that given in test meal, considering only the total fatty acid mass and total radioactivity in the lymph and in the diet.

In all patients the relative specific radioactivity of the triglyceride fatty acids was maximal between 6 and 8 hours after the administration of the labeled fatty acids. In Figure 2 the time course of the absorption of fatty acids of exogenous and endogenous origin has been calculated from the data given in Figure 1. In Patients 3 and 4 the lymph contained an increased amount of endogenous fatty acids at the peak of fat absorption. In Patients 1 and 2 there was a tendency toward an increased amount of endogenous fatty acids 2 to 6 hours after the peak of exogenous fatty acids in the lymph. The total amount of endogenous and exogenous lymph triglyceride fatty acids is given in Table VII. It is also clear that 15 hours after the administration of the test meals absorption of labeled exogenous fatty acids was still taking place via the thoracic duct. Inspection of the time course curves in Patients 3 and 4 (Figure 3) gives information about the relationship between the exogenous and endogenous components of the fatty acids of the triglycerides, cholesterol esters, and phospholipids during different stages of the absorption process. In both patients the relative

specific activity of the cholesterol ester fatty acids reached a maximum of about 30%. The relative specific activity of the phospholipid fatty acids, lower than that of the cholesterol ester fatty acids, was rarely above 20%.

Table VI permits comparison of the analytical data from the dietary fatty acids with the figures from the lipid classes of the thoracic lymph. The fatty acid composition of the lymph triglycerides was very similar to that of the fed fat. However, the relative per cent of stearic acid was less than in the test meal. In the cholesterol ester fractions palmitic and stearic acids were both found in a lower relative concentration than in the test meal. The composition of the phospholipid fatty acids was different from that of the dietary fatty acids.

The distribution of radioactivity among the fatty acids of cholesterol esters, triglycerides, and phospholipids in Patients 3 and 4 shows that every lipid class had a distinct distribution pattern.

In the triglyceride fraction analyzed, the distribution of radioactivity was very similar to that in the test meal in Patient 3. In Patient 4, where stearic acid was added to the diet, somewhat less radioactivity was recovered in stearic acid relative to the other fatty acids. In every cholesterol ester sample analyzed in Patients 3 and 4 relatively more radioactivity was found in oleic acid than in any of the other fatty acids being studied. The distribution of the radioactive fatty acids in phospholipids (Patient 3) and lecithins (Patient 4) showed a strong specificity for linoleic acid

TABLE VI

*Distribution of mass and radioactivity and specific activities among the fatty acids of the test meals and of the cholesterol esters, triglycerides, and phospholipids of the thoracic duct lymph**

Time after test meal	Mass distribution				Radioactivity distribution				Specific activities			
	16:0	18:0	18:1	18:2	16:0	18:0	18:1	18:2	16:0	18:0	18:1	18:2
hours	%				%				*counts/sec/mg*			
					Patient 3							
					Dietary fatty acids							
	34.6		30.1	35.3	34.6		31.5	33.9	183.9		183.6	183.7
					Cholesterol esters							
6	16.5	1.9	37.3	36.8	22.5		50.3	27.2	43.1		42.6	23.4
8	20.0	0.8	43.5	28.4	22.3		50.3	27.4	75.2		77.9	65.1
9	19.8	2.2	42.0	29.0	22.1		49.5	28.4	43.5		60.8	50.5
10	19.3	1.8	38.2	33.7	21.8		52.0	26.2	58.8		70.8	40.4
					Triglycerides							
6	27.4	1.7	36.0	31.6	28.2		37.6	34.2	106.7		108.3	112.2
8	29.5	1.4	38.8	29.4	29.7		37.5	32.8	155.1		149.4	171.8
9	26.7	1.6	37.3	33.4	28.7		36.9	34.4	144.2		132.7	138.3
10	31.7	1.1	33.7	31.0	27.0		36.7	36.3	102.2		130.7	140.5
					Phospholipids							
8	30.0	19.9	12.0	31.9	13.8		22.7	63.5	13.9		57.5	60.5
9	28.6	16.9	10.3	33.0	17.4		14.8	67.8	13.4		31.8	45.5
					Patient 4							
					Dietary fatty acids							
	26.4	24.4	24.6	24.6	25.5	24.4	25.2	24.9	168.5	177.0	176.9	175.0
					Cholesterol esters							
7	19.3	8.4	37.4	25.4	15.4	21.0	43.9	19.7	48.4	151.5	70.7	46.7
8	19.7	8.2	38.7	30.5	17.7	17.4	44.1	20.7	64.3	151.2	81.5	48.5
					Triglycerides							
7	28.3	12.3	30.9	28.5	22.1	16.0	31.6	30.3	106.7	180.4	138.5	143.6
8	28.0	15.0	30.2	25.9	25.2	17.6	30.7	26.5	121.3	160.3	137.0	138.7
					Lecithins							
7	28.2	15.2	8.6	36.5	11.0	21.8	15.5	51.6	9.2	33.6	43.1	33.0

* In Patient 4 the lymph lecithins were isolated and analyzed. Values are expressed as percentage of total fatty acid methyl esters and percentage of radioactivity.

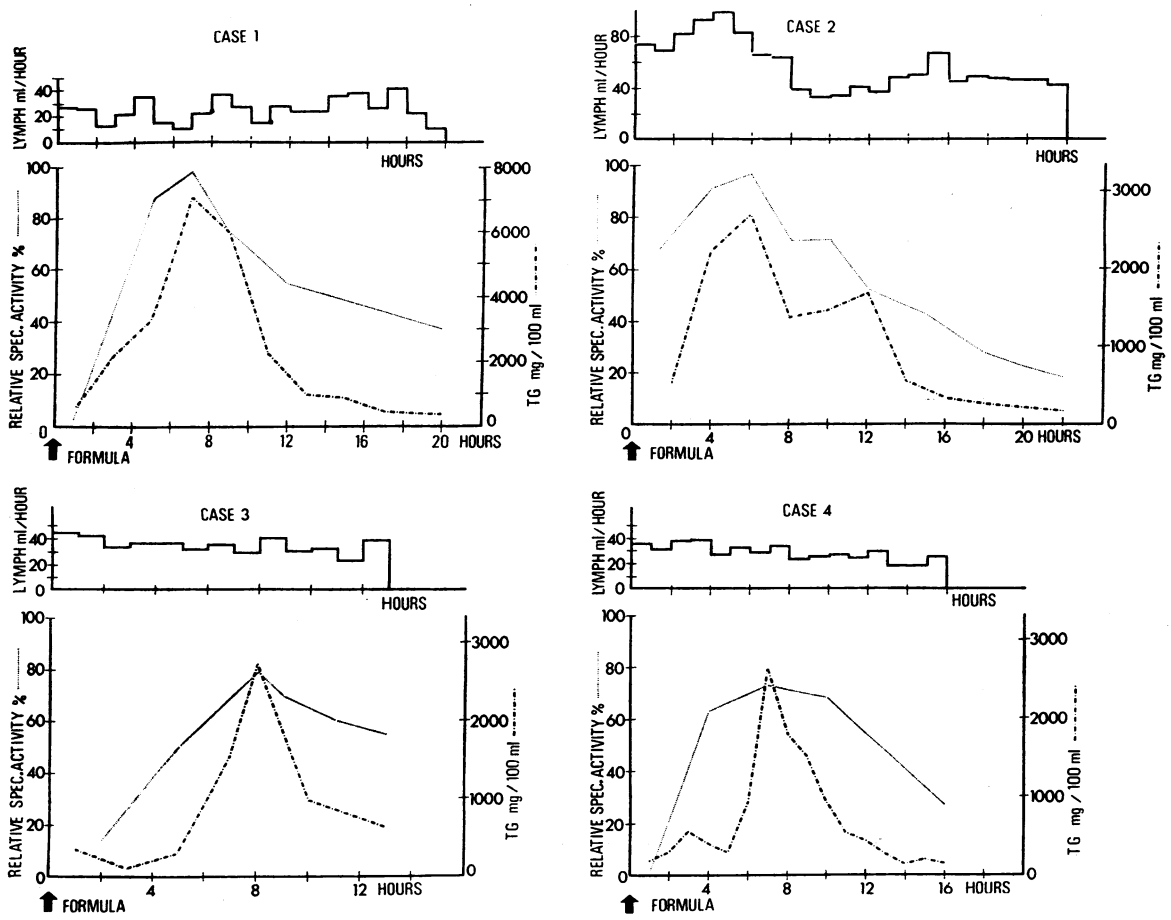

FIG. 1. TIME COURSE CURVE SHOWING SPECIFIC RADIOACTIVITY OF LYMPH TRIGLYCERIDE (TG) FATTY ACIDS (CONSIDERED EN BLOC) RELATIVE TO THAT OF FATTY ACIDS IN THE TEST MEALS ADMINISTERED TO THE FOUR PATIENTS. The concentration of triglycerides and the lymph volumes are recorded.

relative to the other fatty acids tested. The values of the specific radioactivity of each fatty acid in each of the lipid classes compared to those of the same fatty acids in the test meal are shown in Table VIII. These analytical data indicate how much the dietary fatty acids have been diluted with endogenous fatty acids in each lipid class.

From these data it is possible to get information about the composition of the endogenous fatty acids in various lipid classes by subtraction from the total fatty acids the exogenous fatty acid mass and distribution. The composition of the endogenous fatty acids at the peak of fat absorption in Patients 3 and 4 is shown in Table IX. Oleic acid was prominent in the cholesterol esters in both cases. In Patient 3 palmitic acid constituted 46.8% of the total endogenous fatty acids in the triglycerides, but in Patient 4 oleic acid was the most dominating endogenous fatty acid.

Discussion

The metabolic interrelations of fatty acids during the process of intestinal absorption can be stud-

TABLE VII

Total amount of exogenous and endogenous fatty acids during 12 hours in human thoracic duct lymph triglycerides after feeding different test meals (Table I)

Patient	Exogenous fatty acids in lymph triglycerides	Endogenous fatty acids in lymph triglycerides	Endogenous fatty acids/ Total triglyceride fatty acids
	mg	*mg*	%
1	6,731	2,284	25.3
2	10,593	2,217	17.3
3	2,339	1,253	34.9
4	1,947	1,078	35.6

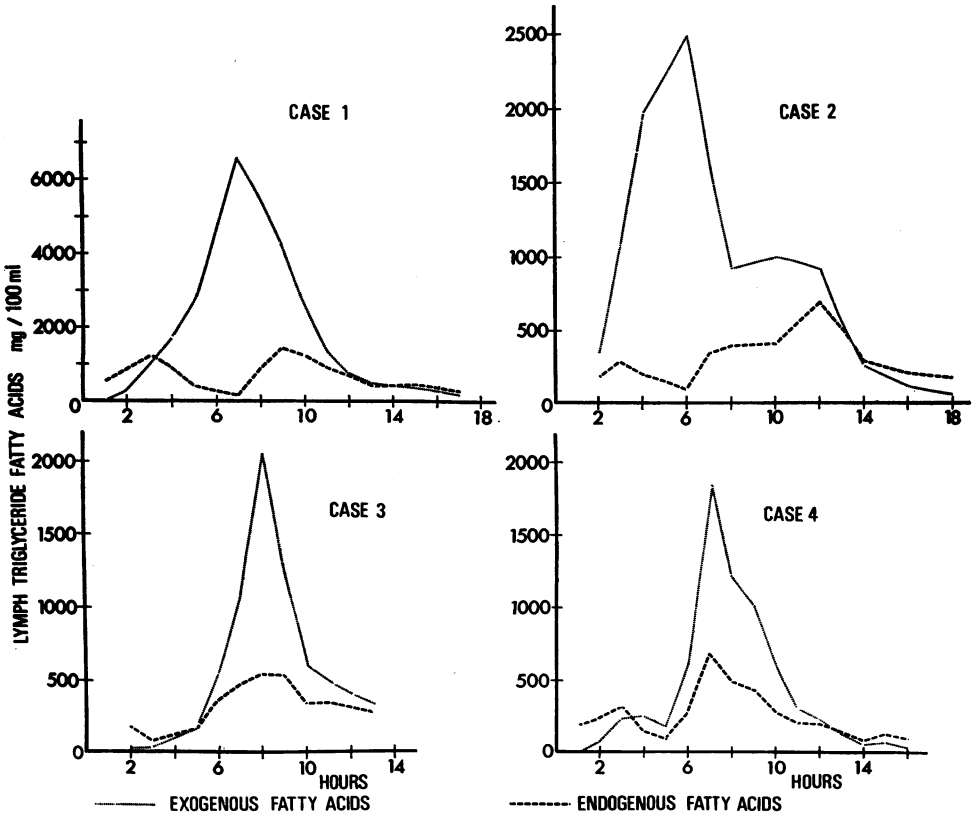

FIG. 2. RELATIONSHIP BETWEEN EXOGENOUS AND ENDOGENOUS FATTY ACIDS OF LYMPH TRIGLYCERIDES AS CALCULATED FROM THE ISOTOPIC DATA IN FIGURE 1 DURING THE ABSORPTION.

ied in human lymph, via thoracic duct cannulation, by a combination of gas-liquid chromatography and radioactive tracer technique, as first shown by Blomstrand, Dahlbäck, and Linder (6, 7). These authors noted a tendency towards specific incorporation of stearic acid, as distinct from palmitic, oleic, and linoleic acids, into lymph phospholipids. Their investigations also disclosed a high degree of specificity for the fatty acid distribution in human lymph lecithins, a finding in ac-

TABLE VIII

Relative specific radioactivity of each fatty acid in each lipid class expressed as per cent of each dietary fatty acid

Time after test meal	Relative specific radioactivity							
	Patient 3				Patient 4			
	16:0	18:0	18:1	18:2	16:0	18:0	18:1	18:2
hours								
				Cholesterol esters				
6	23.4		23.2	12.7				
7					28.7	85.6	40.0	26.7
8	40.9		42.4	35.4	32.2	85.4	46.1	27.7
9	23.7		33.1	27.5				
10	32.0		38.6	22.0				
				Triglycerides				
6	58.0		59.9	61.1				
7					63.3	101.9	78.3	82.1
8	84.3		81.4	93.5	72.0	90.6	77.5	79.3
9	78.4		72.3	75.3				
10	55.6		71.2	76.5				
				Phospholipids*				
7					5.5	19.0	24.4	18.9
8	7.6		31.3	32.9				
9	7.3		17.3	24.8				

* In Patient 4 the lecithin was analyzed.

TABLE IX

*Distribution of endogenous fatty acids among the fatty acids of the cholesterol esters, triglycerides, and phospholipids (lecithins) of the thoracic duct lymph**

Patient		16:0	18:0	18:1	18:2
3	Cholesterol esters	21.2	1.7	34.1	28.5
	Triglycerides	46.8		30.2	23.0
	Phospholipids	30.8	14.3	7.6	34.1
4	Cholesterol esters	18.8	1.4	39.7	29.1
	Triglycerides	28.9	8.7	44.9	11.9
	Lecithins	33.2	23.9	9.9	25.7

* The lymph samples analyzed were collected at the peak of fat absorption. The composition of the endogenous fatty acids was calculated from the total minus the exogenous fatty acid mass and distribution.

cord with observations made by Hanahan and Blomstrand in rats (24).

In the present investigation, which is a continuation of those studies, we fed a test meal of three or four ^{14}C-labeled fatty acids to patients with cannulated thoracic ducts, then analyzed each lymph lipid class by means of gas-liquid radiochromatography as described in a preliminary communication (11). A similar experimental procedure was recently used by Karmen and his associates in an extensive investigation on rats (9, 10).

The data obtained in the present study throw light on two factors, the fatty acid specificity for each lymph lipid class, and the ratio between the endogenous and exogenous moieties of each fatty acid within each lymph lipid class.

The distribution of total lymph fatty acids among the different lipid classes after each test

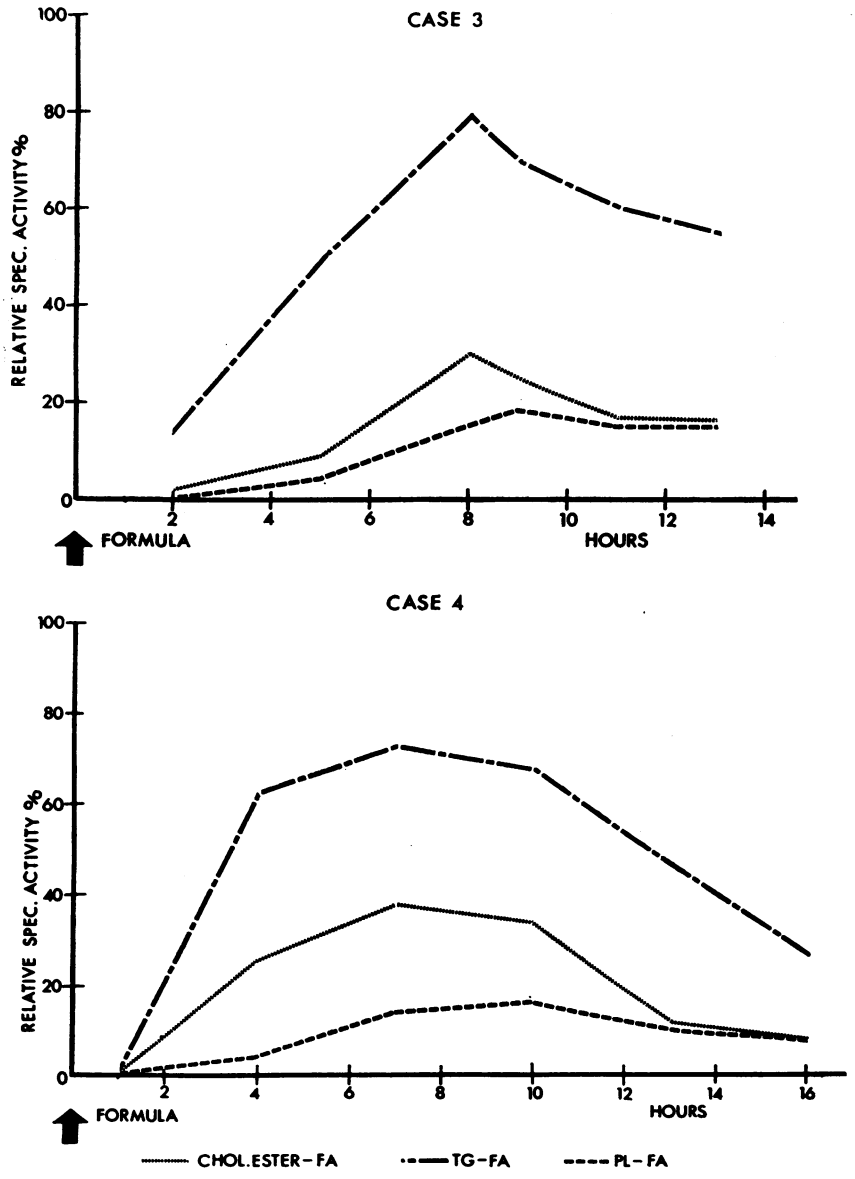

FIG. 3. RELATIVE SPECIFIC ACTIVITIES OF FATTY ACIDS (FA) OF LYMPH TRIGLYCERIDES (TG), CHOLESTEROL ESTERS, AND PHOSPHOLIPIDS (PL) (CASE 4, LECITHINS) DURING THE ABSORPTION OF FREE FATTY ACID ADMINISTERED TO CASES 3 AND 4.

meal administered in Patients 3 and 4 is shown in Table V. For each lipid class both the fatty acid distribution and the radioactivity distribution patterns were in accord with previous analytical results for human thoracic duct lymph (6, 7).

By plotting time curves for the fatty acids in Patient 3 we found that palmitic, oleic, and linoleic acids were absorbed in equal degree (Table VI). During the absorption process the dietary fatty acids, as pointed out by Karmen and co-workers (9, 10), mix with endogenous fatty acids in the intestinal lumen and mucosa to form a pool that is available for the formation of chylomicron esters. If the mechanism involved in chylomicron ester formation shows affinity for one fatty acid relative to another, then relatively more of the fatty acid will be incorporated into the ester. The final distribution of radioactivity among the fatty acids in each lipid class will then reveal a bias in that direction even though the fatty acids are absorbed in equal degree.

Table VI compares the distribution of radioactivity among the fatty acids of each lymph lipid class with the distribution in the test meal. From these data it is apparent that each lipid class has a very distinctive radioactivity distribution pattern.

The distribution of radioactivity in the lymph triglyceride fraction was very similar to that in the test meal. The only exception was stearic acid (Table VI, Patient 4), which was incorporated into the lymph triglycerides somewhat less effectively than the other three fatty acids studied. With regard to palmitic, oleic, and linoleic acids the process of lymph triglyceride formation was nonspecific and did not distinguish among these fatty acids.

Analyses of the data in Table VI indicate that labeled oleic acid was relatively more abundant in the cholesterol esters, as could be expected from the test meal. It is clear from these results that the process of lymph cholesterol ester formation during fat absorption showed far greater affinity for dietary oleic acid than for the other fatty acids studied. The fatty acid percentages in the lymph cholesterol esters were similar in Patients 3 and 4, as was the radioactivity distribution for palmitic, oleic, and linoleic acids.

The most distinct fatty acid radioactivity pattern was that of the phospholipid class, where linoleic acid was very selectively incorporated by comparison with palmitic, stearic, and oleic acids (Table VI). This conflicts with an earlier observation (6) that labeled stearic acid dissolved in oil and administered singly showed preferential incorporation into human lymph phospholipids. A possible explanation may be the difference of the metabolic design between the present and previous studies. A detailed comparison indicates that in earlier studies (6) in human beings the patients were not fasting but on a general mixed diet when fed the labeled fatty acids. In this study the patients were fasting overnight when fed the rather extreme formula meal containing the dietary fat in the form of free fatty acids. This difference in nutritional status and test meal composition might explain the conflicting results; further studies with other diets are therefore necessary.

Specific activity of each fatty acid can be calculated from the amounts of radioactivity recovered in each ester class and the fatty acid composition of each lipid class (Table VI). Comparison with the specific activity of each dietary fatty acid will give the relative specific activity (Table VIII). From the relative specific radioactivity of each fatty acid in a lipid class, the exogenous and endogenous moieties of the fatty acid can be calculated (Figure 2).

It is evident from Tables VII and VIII that in each lipid class the ratios between the exogenous and endogenous moieties of the fatty acids varied. Another interesting observation is that the percentage of total triglyceride fatty acids that was endogenous was higher (about 35%) in Patients 3 and 4 than in Patients 1 and 2 (25.3 and 17.3, respectively). In the former two patients only free fatty acids were given.

Within each lymph lipid class the extent of endogenous dilution varied among the fatty acids (Table VIII). In all the lymph samples analyzed in Patient 3 the endogenous moiety of palmitic, oleic, and linoleic acids for each of the fatty acids of the cholesterol esters was greater than the exogenous moiety. In Patient 4 where stearic acid was included in the dietary labeled acids the endogenous moiety was greater for each of the fatty acids of the cholesterol esters except stearic acid, where the exogenous moiety was significantly greater. In the phospholipids the major part of each fatty acid was endogenous (Table VIII). At the peak of fat absorption substantial amounts of

endogenous fatty acids were used for synthesis of the lymph lipid classes.

Our results are thus consistent with the rat experiments of Karmen and co-workers (9, 10), who observed that during the process of fat absorption a considerable quantity of endogenous fatty acids evidently plays an important role in the synthesis of lipid classes in the intestinal mucosa. Two possible pathways for triglyceride biosynthesis in the intestinal mucosa have been reported in animal experiments (25–27). One employs β-monoglyceride for the condensation reaction, and the other employs α-glycerophosphate as the precursor of the triglyceride. The metabolic interrelations between the triglycerides and the lecithins in the chylomicrons have been critically discussed by Weiss, Kennedy, and Kiyasu (28).

In a recent report by Whyte, Goodman, and Karmen (29) on the specific distribution of fatty acids in the chylomicron triglycerides and lecithins in the rat, the important point was made that the incorporation of exogenous labeled fatty acids into different positions was nearly random in the triglycerides but markedly nonrandom in the lecithins, where saturated acids, especially stearic acid, were predominantly esterified at the α-position and polyunsaturated fatty acids at the β-position. These findings thus agree with the reported asymmetric incorporation of stearic acid and linoleic acid into human chylomicron lecithins in our earlier studies (6).

Preliminary analyses of the positional relations of the fatty acids in the triglycerides and lecithins of lymph in the present study strongly support the nonrandom incorporation of exogenous fatty acids into the lymph lecithins. A more extensive discussion about the mechanism of lymph triglyceride formation in man must, however, await a detailed analysis of the distribution of exogenous and endogenous fatty acids in the triglyceride molecule.

Further studies on the structure of lymph triglycerides and lecithins under different dietary conditions would doubtless help to clarify the ratio between endogenous and exogenous fatty acids. Such analyses will also reveal the interrelationship between the triglycerides and lecithins during the synthesis in the intestinal mucosa.

The specificity of the esterifying enzymes and the dilution of the exogenous fatty acids with endogenous fatty acids reveal the great complexities of the mechanisms involved in fat absorption.

At the moment it is difficult to assess the metabolic significance of the observation that chylomicron cholesterol esters showed a preferential utilization of oleic acid in cholesterol esterification. It is, of course, possible that the observed fatty acid specificity for chylomicron cholesterol ester formation affects the general cholesterol metabolism. Further studies in patients with hypercholesterolemia with a technique similar to that used in the present investigation are strongly indicated.

Summary

Investigations were conducted in patients with cannulated thoracic ducts in order to study the mechanisms involved in the formation of cholesterol esters, triglycerides, and phospholipids during fat absorption after feeding a single formula meal containing one single ^{14}C-labeled fatty acid or similar amounts of three or four ^{14}C-labeled fatty acids (palmitic, stearic, oleic, and linoleic acids).

The lymph lipids were chromatographed on silicic acid columns to separate cholesterol esters, triglycerides, and phospholipids. After assaying each lymph lipid class for total radioactivity, gas radiochromatography was employed to measure the distribution of mass and radioactivity in the individual fatty acids of each lipid class.

In the processes of fatty acid absorption and lymph triglyceride formation no specificity for one fatty acid relative to another was observed comparing palmitic, oleic, and linoleic acids. A slight discrimination against stearic acid was observed in absorption and the formation of lymph triglycerides.

During fatty acid absorption lymph cholesterol ester formation showed marked specificity for oleic acid relative to other fatty acids tested.

Linoleic acid was selectively incorporated into lymph phospholipids by comparison with palmitic, stearic, and oleic acids.

During the fat absorption a considerable amount of endogenous fatty acids was used for synthesis of the various lipid classes of the lymph.

Appendix

Case reports

Patient 1 (*P.G.-00*), a 62-year-old woman, had a malignant melanoma on the back of her left hand that had

been excised about 1 year earlier. One month before admission, enlarged epitrochlear and axillary lymph nodes had been found, and needle aspiration biopsy disclosed malignant cells. Chest X rays revealed no metastases. There was no history of gastrointestinal symptoms or abnormalities. Coincident with scalene lymph node biopsy, the thoracic duct was cannulated under general anesthesia (Thiopentone and succinylcholine were used to induce anesthesia for endotracheal intubation, light ether and nitrous oxide anesthesia for maintenance; respiration was spontaneous). Seven hours after cannulation the patient was given a formula diet (see Table I). Lymph samples were collected hourly for the next 20 hours during this study. Since the scalene lymph node biopsy was positive for cancer, the plans for cytostatic drug perfusion treatment were abandoned.

Patient 2 (*S.A.-L.-12*), a 50-year-old woman had breast cancer and axillary metastases, but no demonstrable distant metastases. There was no history of gastrointestinal symptoms or abnormalities. Coincident with scalene lymph node biopsy the thoracic duct was cannulated under local anesthesia [½% mepivacaine (Carbocaine) with epinephrine 1:200,000]. About 5 hours after cannulation the patient was given a formula diet (see Table I). Lymph samples were collected hourly for the next 22 hours during this study. The biopsy was negative for cancer. About 1 week later, radical mastectomy was performed.

Patient 3 (*S.T.-15*), a 48-year-old woman, had undergone radical mastectomy and oophorectomy for breast cancer with axillary metastases 3 years earlier. A local skin recurrence has recently been diagnosed by needle aspiration biopsy and treated by local excision. Roentgen examination disclosed pulmonary metastases but no skeletal metastases. There was no history of gastrointestinal dysfunction. Serum alkaline phosphatase activity was about 4 U per ml (Bessey-Lowrey), but other laboratory findings showed no signs of hepatobiliary disease. Hepatic scintillography as well as selective angiography of the celiac artery was normal, and no metastases could be demonstrated. In connection with scalene lymph node biopsy the thoracic duct was cannulated under local anesthesia (½% mepivacaine). Next morning (25 hours after cannulation), the patient was given a formula diet (see Table I). Lymph samples were collected hourly for the next 13 hours during this study.

Patient 4 (*A.P.-90*), a 74-year-old man, had been admitted for suspected esophageal neoplasia after 5 months dysphagia for solid food and weight loss of 3 kg. There was no history of gastrointestinal dysfunction. Coincident with scalene lymph node biopsy to check on cervical metastases, the thoracic duct was cannulated under local anesthesia (½% mepivacaine with epinephrine 1:200,000). About 5 hours after cannulation the patient was given a formula diet (See Table I). Lymph samples were then collected hourly for the next 16 hours during this study. Twelve days after cannulation, laparotomy disclosed a tumor of the cardiac orifice which, on microscopic examination, was found to be an adenocarcinoma. At gastroesophageal resection both the liver and other abdominal organs appeared to be free from metastases.

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